

**FIG. 1**

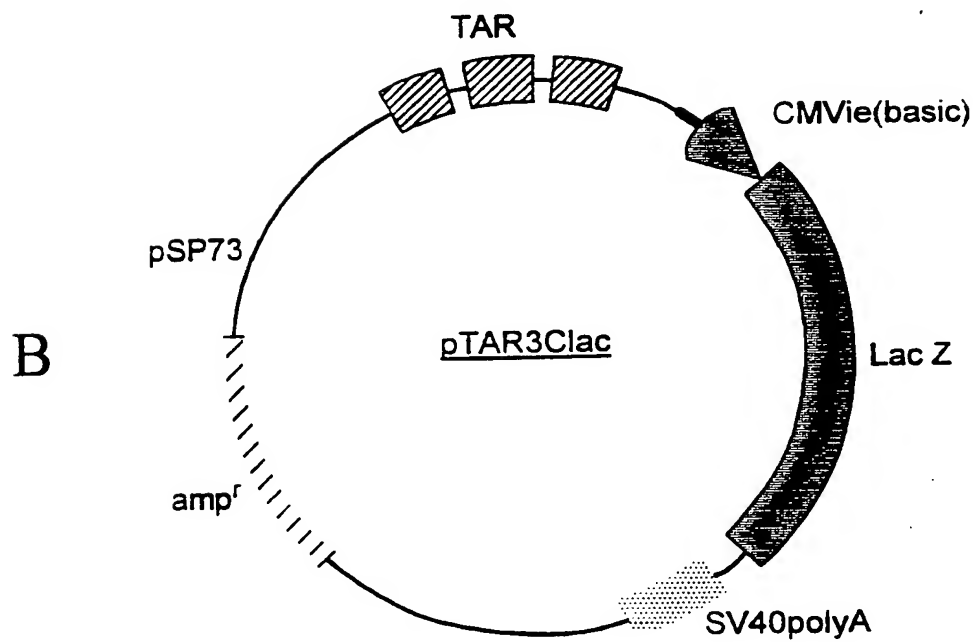
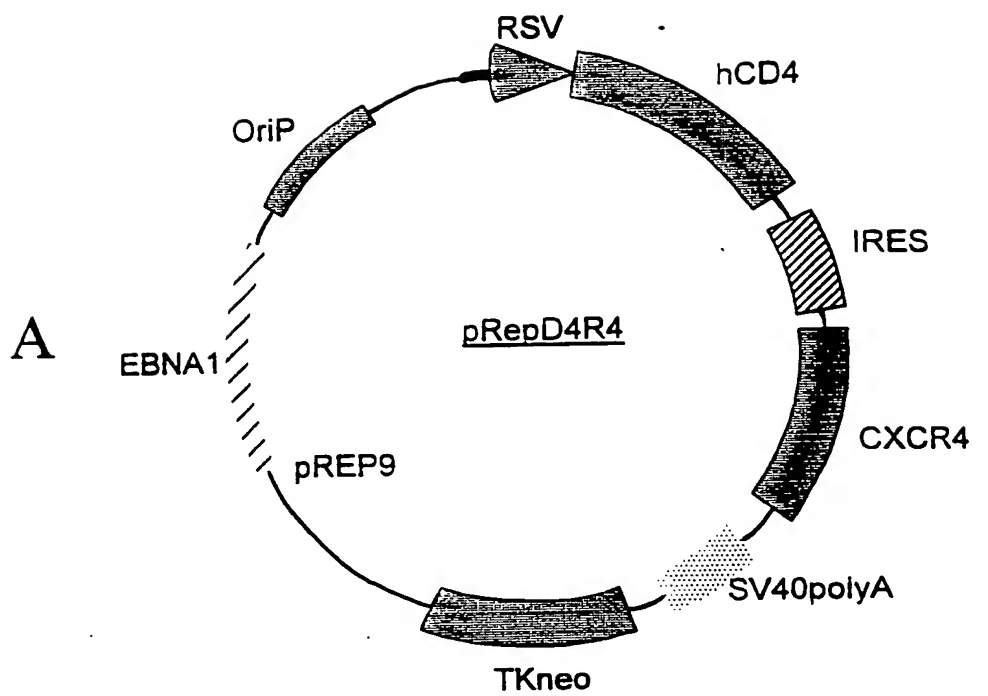


FIG. 2

FIG. 3

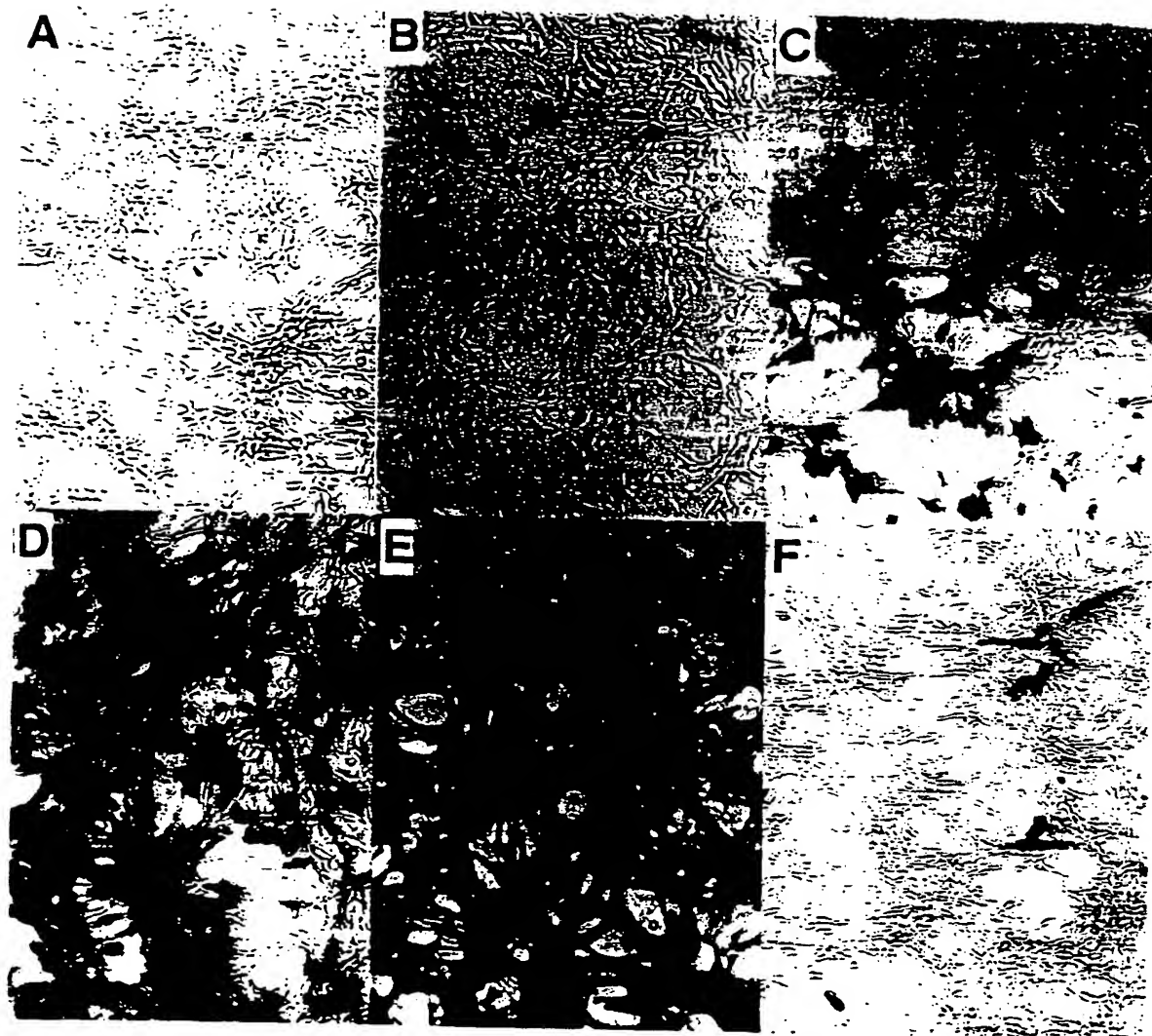


FIG. 4A

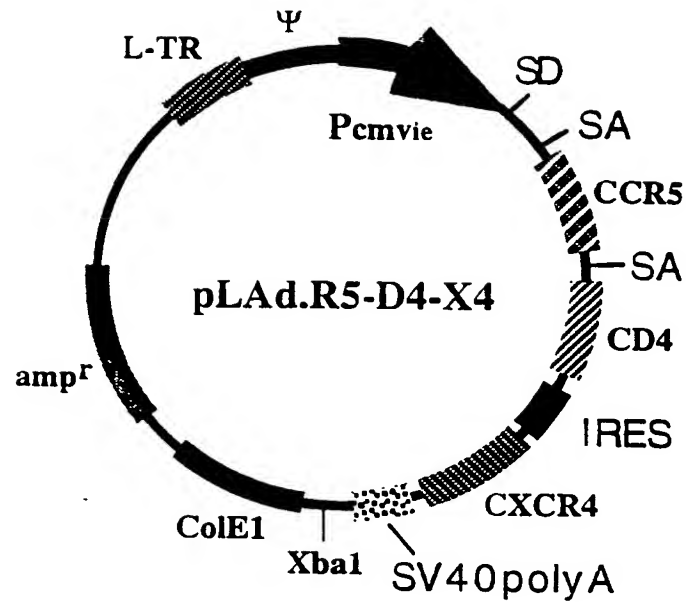


FIG. 4B

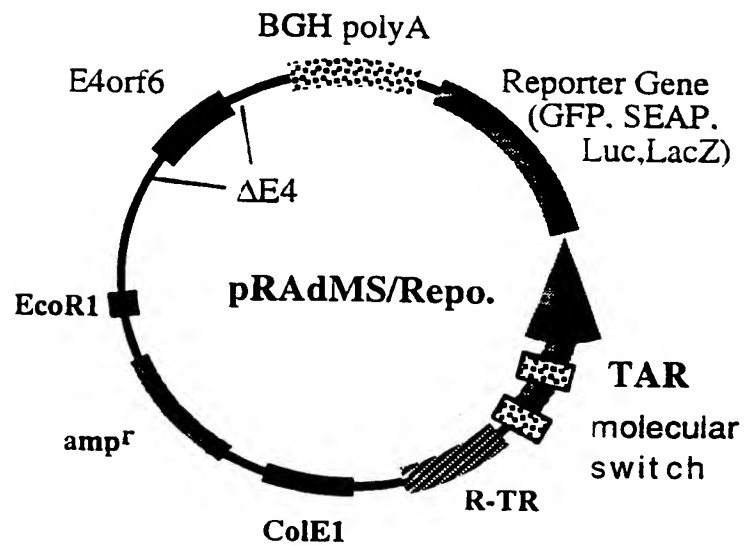


FIG. 5A

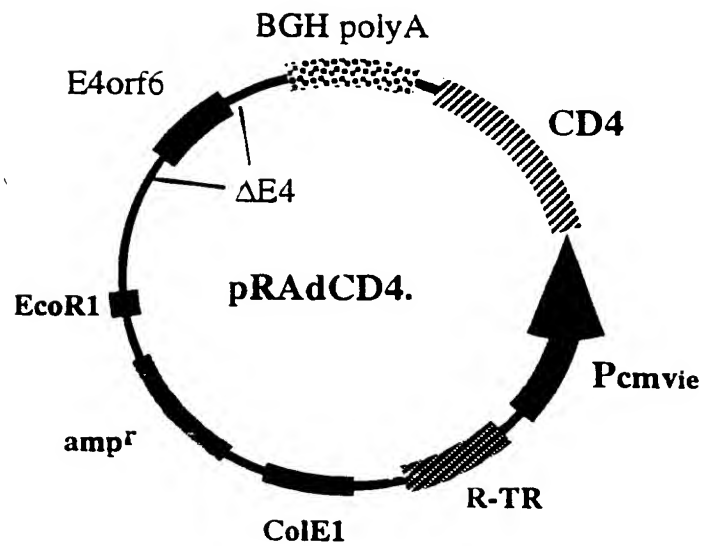
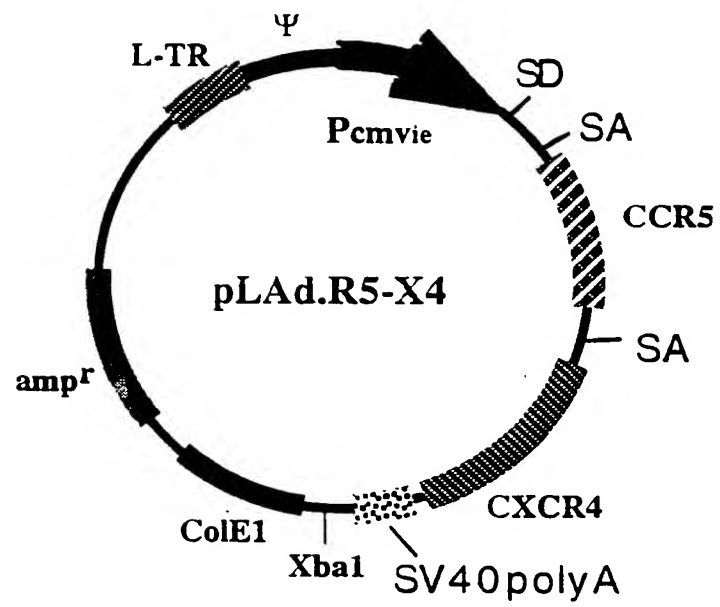


FIG. 5B

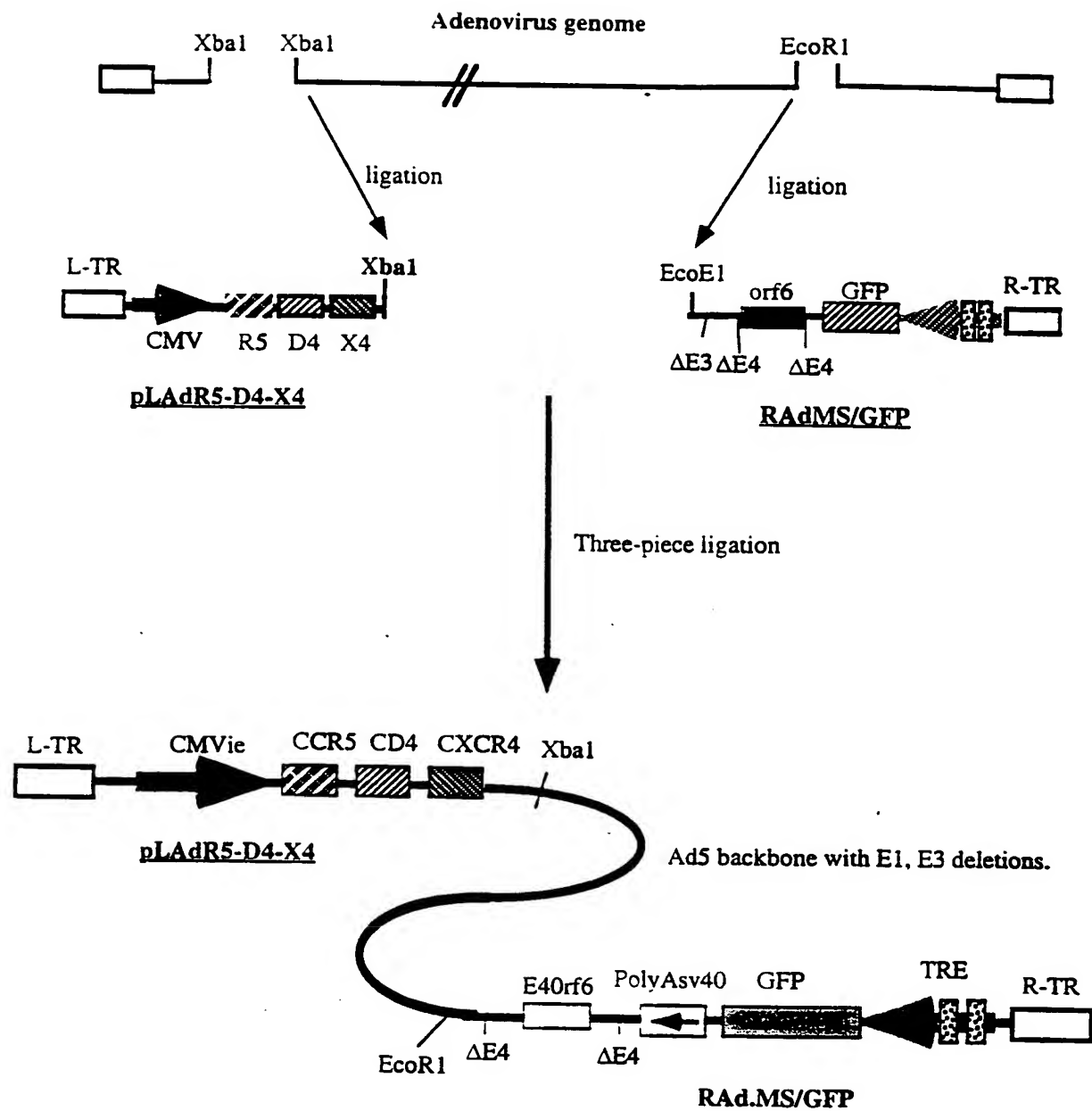


FIG. 6

# pLAd-CCR5.CXCR4 (9 Kb)

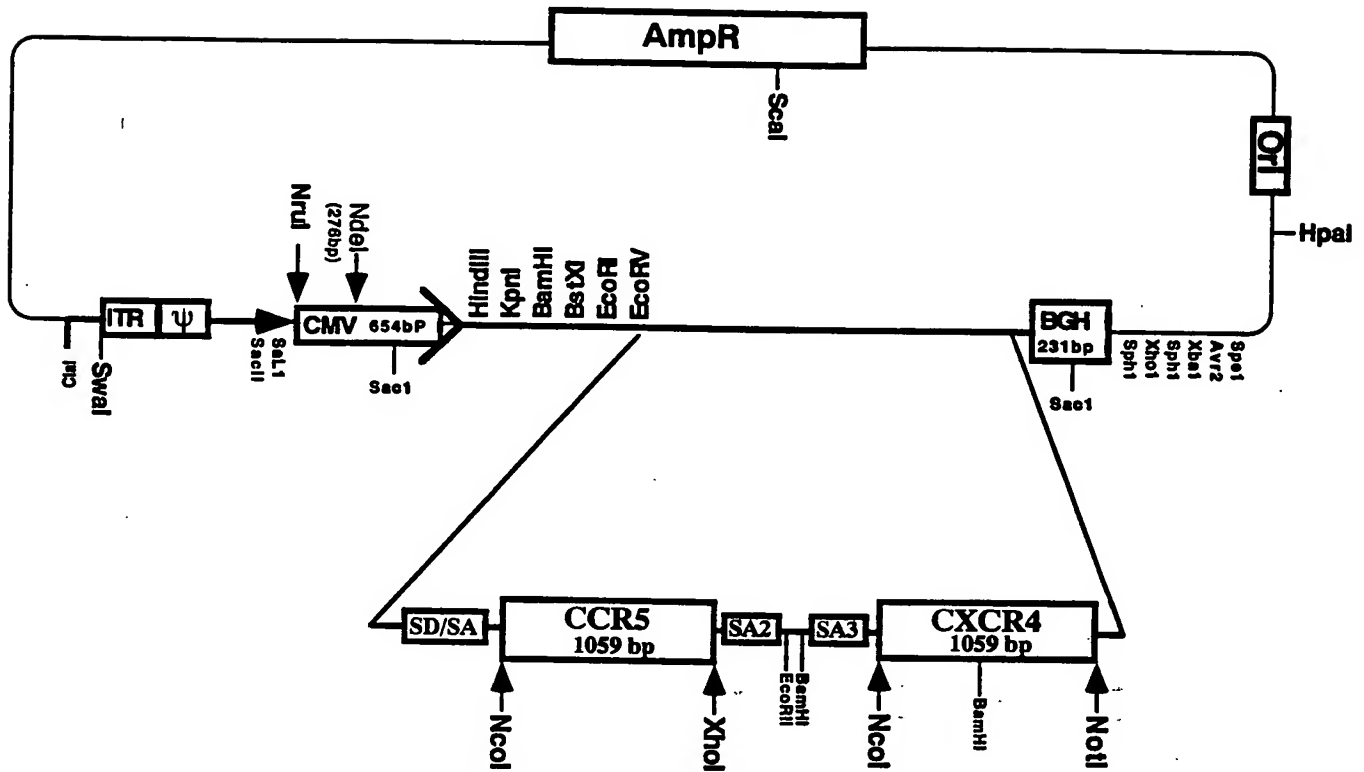


FIGURE 7A

# pRAd.CMV.Fiber.ORF6-CD4.CXCR4 (~14.8 Kb)

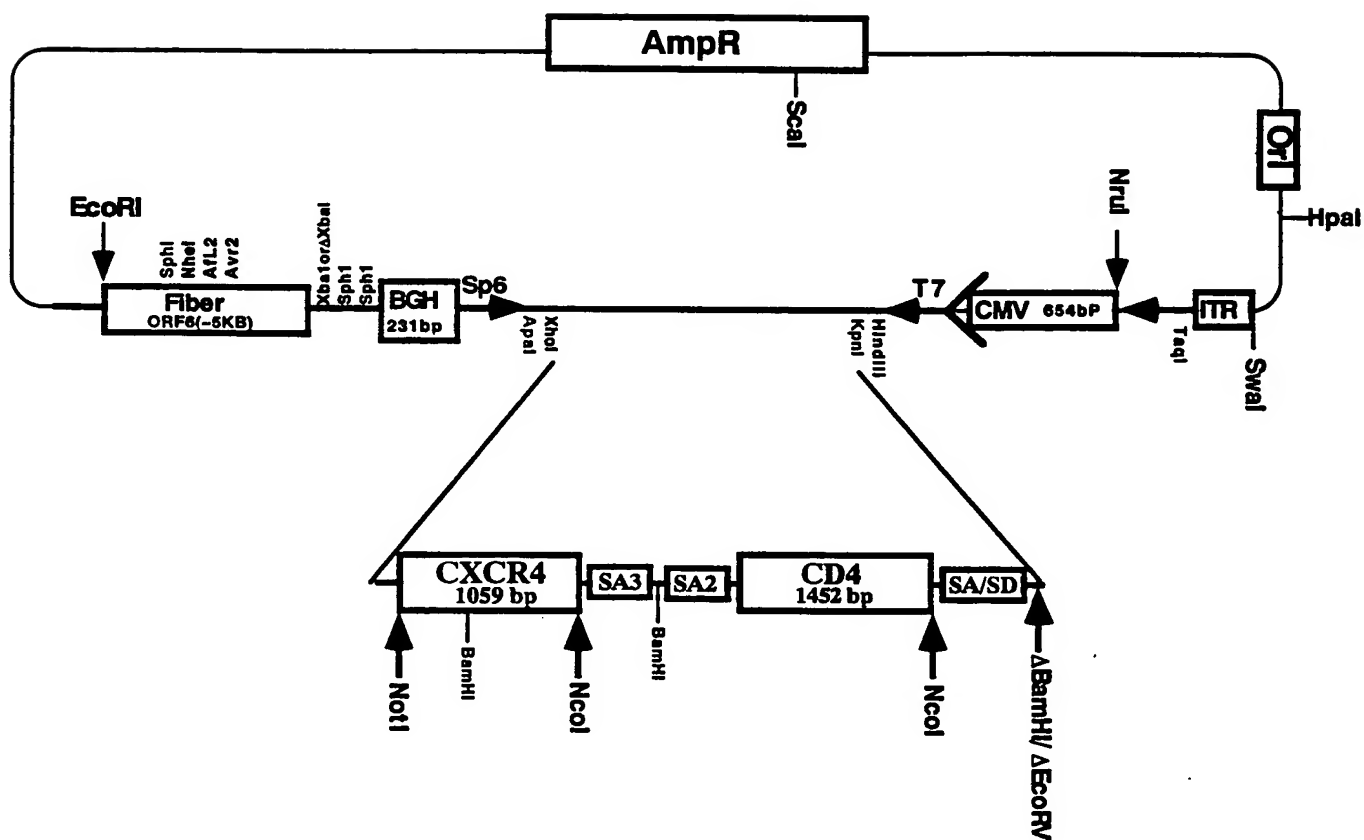
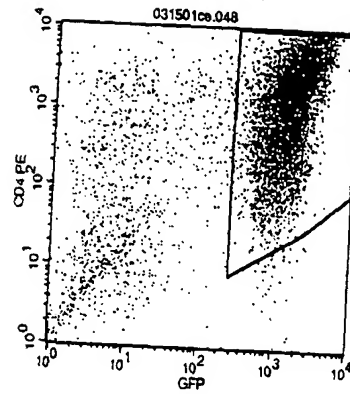


FIGURE 7B



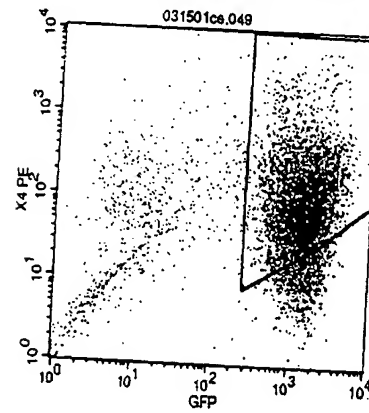
**FIGURE 8**

**A.**



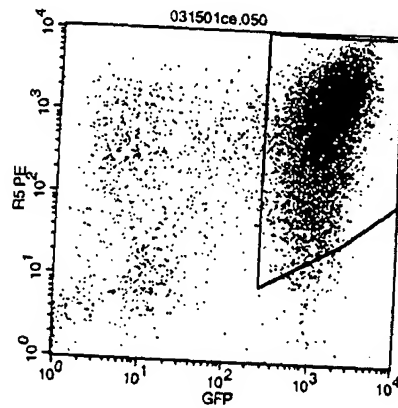
CD4 Expression Level: 84.0 %

**B.**



CXCR4 Expression Level: 77.7 %

**C.**



CCR5 Expression Level: 89.6 %

FIGURE 8 D



E



F



**FIGURE 8G**

#	Cell Types	Infected	Fluorescent-PE Ab	All %Gated	M1 %Gated	All Mean	M1 Mean	All Geo Mean	M1 Geo Mean
1	HUT78	non	mouse IgG 2ak-PE	100	1.2	4.04	21.74	3.35	18.83
2		non	CD4-PE	100	99.92	628.77	629.25	484.56	486.22
3		non	CXCR4-PE	100	65.29	24.21	32.1	18.27	26.74
4		non	CCR5-PE	100	1.56	4.14	27.48	3.34	23.44
5	CEM-NKr-R5	non	mouse IgG 2ak-PE	100	3.12	3.74	19.8	3.05	16.24
6		non	CD4-PE	100	99.6	343.3	344.67	285.47	290.65
7		non	CXCR4-PE	100	98.99	65.93	66.55	53.8	55.13
8		non	CCR5-PE	100	66.66	38.76	55.64	16.91	32.66
9	Molt-4-R5	non	mouse IgG 2ak-PE	100	1.15	3.87	31.78	3.31	20.82
10		non	CD4-PE	100	57.1	35.1	57.41	15.58	39.05
11		non	CXCR4-PE	100	91.44	73.1	79.25	47.6	57.08
12		non	CCR5-PE	100	59.66	37.1	57.94	17.75	38.66
13	CEM-A	non	mouse IgG 2ak-PE	100	0.59	3.38	14.07	3.01	12.31
14		non	CD4-PE	100	94.44	98.93	104.5	62.23	73.75
15		non	CXCR4-PE	100	99.69	87.06	87.31	69.86	70.36
16		non	CCR5-PE	100	0.24	3.06	33.62	2.7	17.45
17	MEGI	non	mouse IgG 2ak-PE	100	2.1	6.17	19.43	5.45	18.61
18			CD4-PE	100	96.7	126.69	130.76	97.19	106.47
19			CXCR4-PE	100	50.34	16.25	22.6	14.18	21.27
20			CCR5-PE	100	2.03	6.34	20.31	5.62	19.43
21	Indicator#44	3R122900	mouse IgG 2ak-PE	100	1.86	4.96	27.72	4.16	19.95
22		MOI, 30	CD4-PE	100	95.19	577.67	584.4	271.07	324.48
23			CXCR4-PE	100	95.78	177.72	185.15	108.28	121.08
24			CCR5-PE	100	92.42	258.22	278.69	132.99	167.66
25	Indicator#44	3R122900	mouse IgG 2ak-PE	100	2.08	5.11	32.83	4.21	23.34
26		MOI, 60	CD4-PE	100	93.42	894.83	957.23	393.37	518.33
27			CXCR4-PE	100	93.68	219.61	233.8	125.85	150.97
28			CCR5-PE	100	91.64	369.08	401.96	175.48	232.68

**FIGURE 8G - Cont.**

#	Cell Types	Infected	Fluorescent-PE Ab	All %Gated	M1 %Gated	All Mean	M1 Mean	All Geo Mean	M1 Ge	Mean
29	Hela	non	mouse IgG 2ak-PE	100	1.09	3.85	34.76	3.06		23.76
30		non	CD4-PE	100	1.28	5.05	28.9	4.22		19.37
31		non	CXCR4-PE	100	94.37	69.86	73.42	53.53		59.26
32		non	CCR5-PE	100	2.1	5.29	21.55	4.45		18.95
33	PBMC	non	mouse IgG 2ak-PE	100	0.13	4.02	237.03	2.89		164
34		non	CD4-PE	100	42.6	315.27	731.4	37.45		655.64
35		non	CXCR4-PE	100	20.35	35.15	101.11	18.07		89.8
36		non	CCR5-PE	100	0.24	4.88	316.55	3.03		180.47

**FIGURE 9**

**A.**



**B.**



**C.**



**FIGURE 10**

**Table 1.** Viral infection of Indicator Cells by HIV-1 Subtypes

<b>Cat. No.</b>	<b>Inhibition by IIIB Ab (4 <math>\mu</math>l)</b>	<b>Subtype (gag/env)</b>	<b>Viral Titer (ip/ml)</b>	<b>Co-receptor</b>	<b>NIH p24 (ng/ml)</b>	<b>Phenotype</b>
398	(HTLV-IIIB/H9) 100% (50 ip) 88% (225 ip) 83% (400 ip)	B/B	$2.0 \times 10^5$	X4	-	SI (+++)
1650	(92UG029) 16% (170 ip) 35% (600 ip)	A/A	$8.9 \times 10^3$	X4	47	SI (+)
1996	(93RW002)	/A	$1.9 \times 10^2$	R5	121	SI
2304	(94UG103) 29% (37 ip) 12% (185 ip)	/A	$3.7 \times 10^3$	R5,X4	155	SI (++)
1658	(92TH014)	B/B	$1.2 \times 10^4$	R5	177	SI
2308	(93BR012) 16% (120ip) 38% (660 ip)	/B	$1.2 \times 10^4$	R5	316	SI (+)
1777	(92BR025) 8% (120 ip) 10% (500 ip)	C/C	$1.0 \times 10^4$	R5	164	NSI*( $\pm$ )
4164	(98CN006)	C/C	$8.8 \times 10^3$	R5	160	SI
1684	(92UG005) 23% (400 ip) 22 % (1200 ip)	D/D	$1.7 \times 10^4$	R5	225	SI (+)
1952	(93UG065)	D/D	$1.6 \times 10^3$	X4	245	SI
2166	(93TH053)	/E	$1.4 \times 10^3$	X4	45	NSI*
2167	(93TH054)	/E	$2.4 \times 10^3$	R5	58	SI
2314	(93BR019) 40% (86 ip) 23% (430 ip)	/BF	$8.6 \times 10^3$	X4	128	SI (++)
2329	(93BR020)	F/F	$5.7 \times 10^3$	R5, X4	169	SI
2338	(93BR029) 33% (300 ip)	B/F	$5.8 \times 10^3$	R5	185	SI (+++)
4143	(BCF13) 32% (180 ip)	Group O	$2.9 \times 10^4$	-	-	SI (+++)

All viruses were titrated between day 3 and day 4 postinfection

\* At day 4, there were no syncytial formed.

**FIGURE 11**

Comparison GenPhar Indicator with MAGI Cells								
HIV	HIV Strains	Sources	Catalogue	Co-Receptor	Indicator#44 (lp/ml)	MEGI (lp/ml)	Indicator/MEGI	
Wild type	IIIB	NIH	398	X4	75,000	66,000	1.14	
Patient's Isolates	JM	GenPhar, INC		X4	1,050	490	2.14	
Patient's Isolates	92UG001	NIH	1647	X4, R5	5,200	1,900	2.74	
Patient's Isolates	93TH054	NIH	2167	R5	4,800	0	0.00	
Patient's Isolates	93BR020	NIH	2329	X4R5	5,250	530	9.91	

FIGURE 12

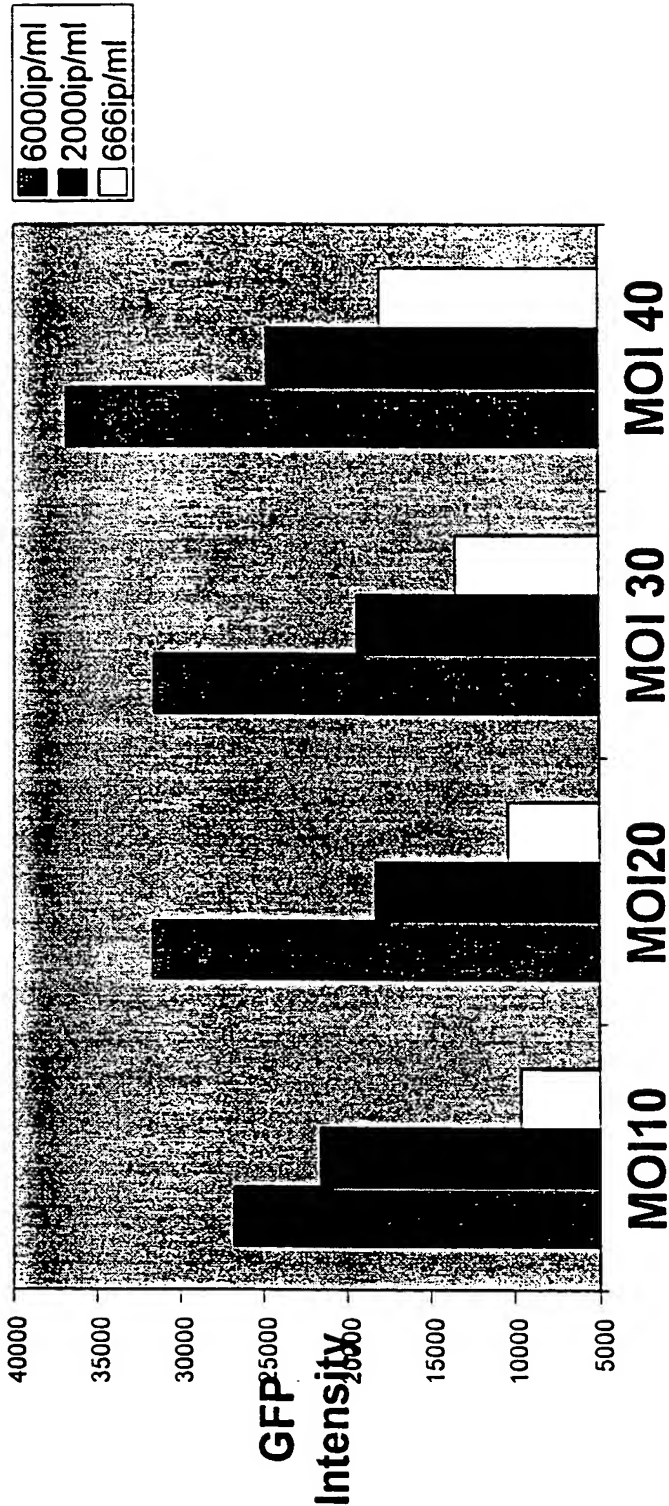
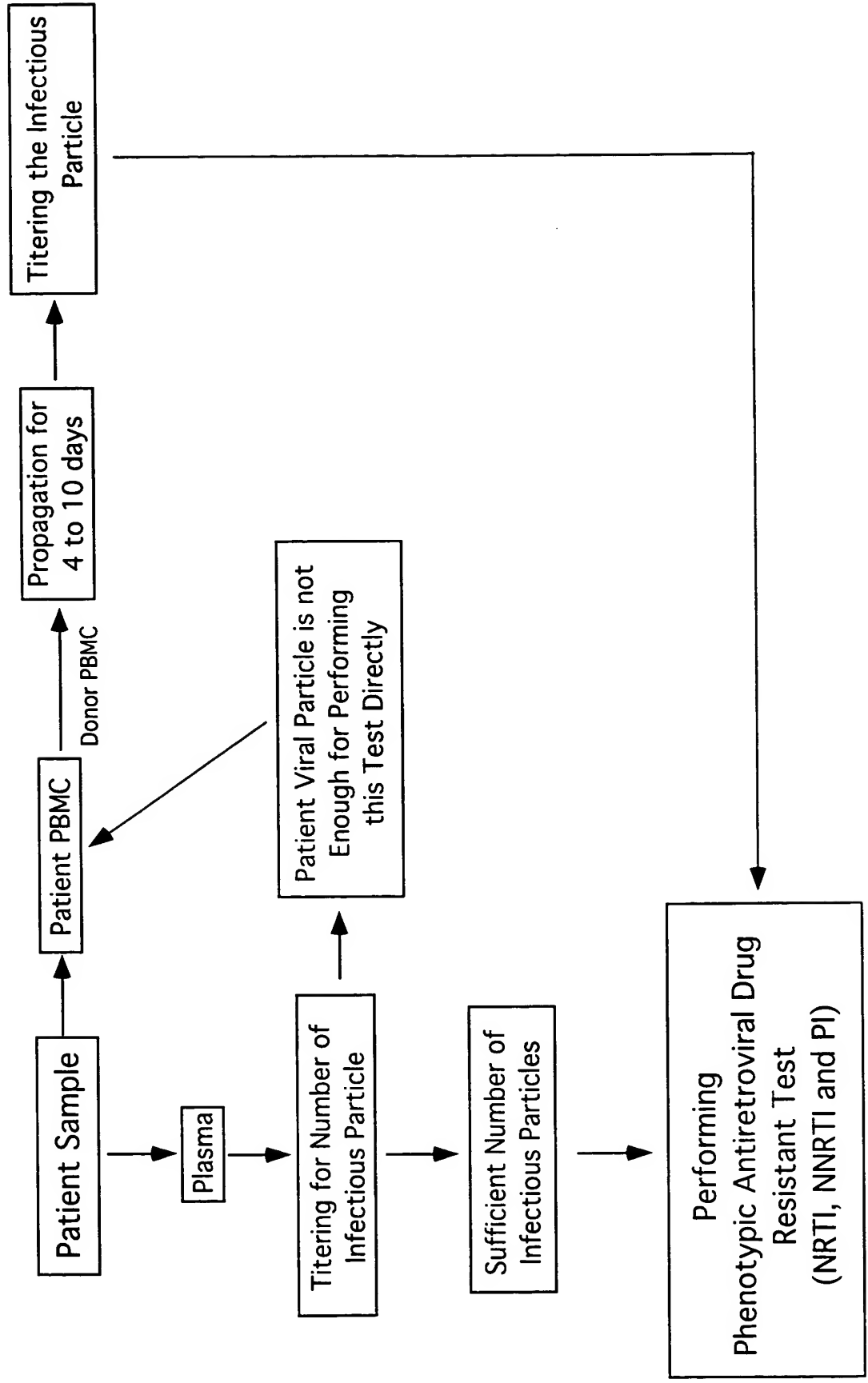


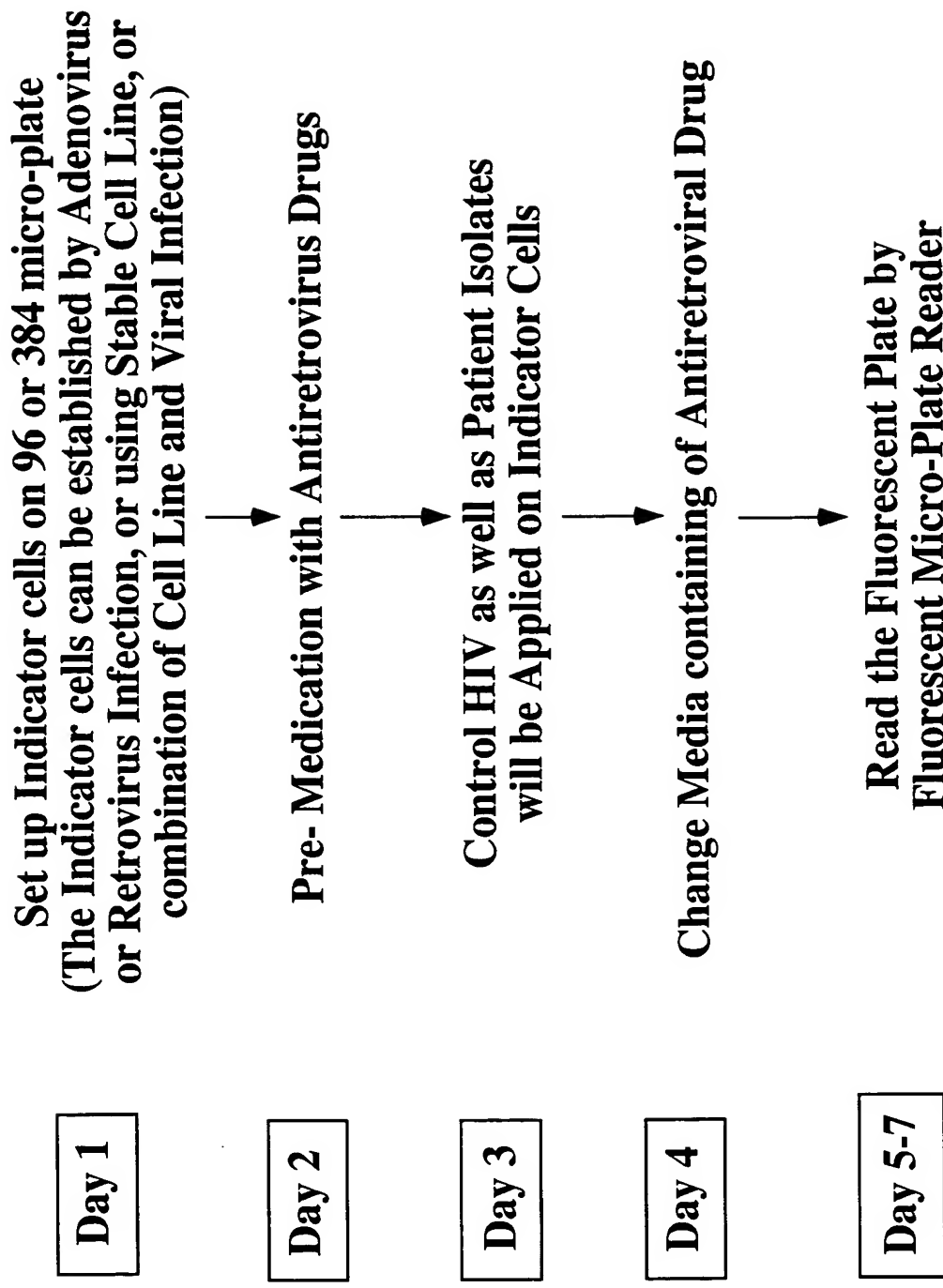


FIGURE 13



**FIGURE 14**

## **Phenotypic Antiretroviral Drug Resistant Test for NRTI and NNRTI**



## **FIGURE 15**

### **HIV DRUG RESISTANCE ASSAY FOR PI**

#### **PRIMARY PLATE**

**Set Up Infection Plate of Cells Containing 3 HIV Receptors with a Sample containing HIV in the Presence of the PI Drug to be tested for a Suitable Period of Time**

#### **SECONDARY PLATE**

**- Day 1      Set Up Indicator Cells Containing 3 HIV Receptors as well as a Reporter Gene for Monitoring HIV Infection**



**Day 1      Transfer the Supernatant of the Culture in the Primary Plate to the Indicator Cells for Titration of HIV**



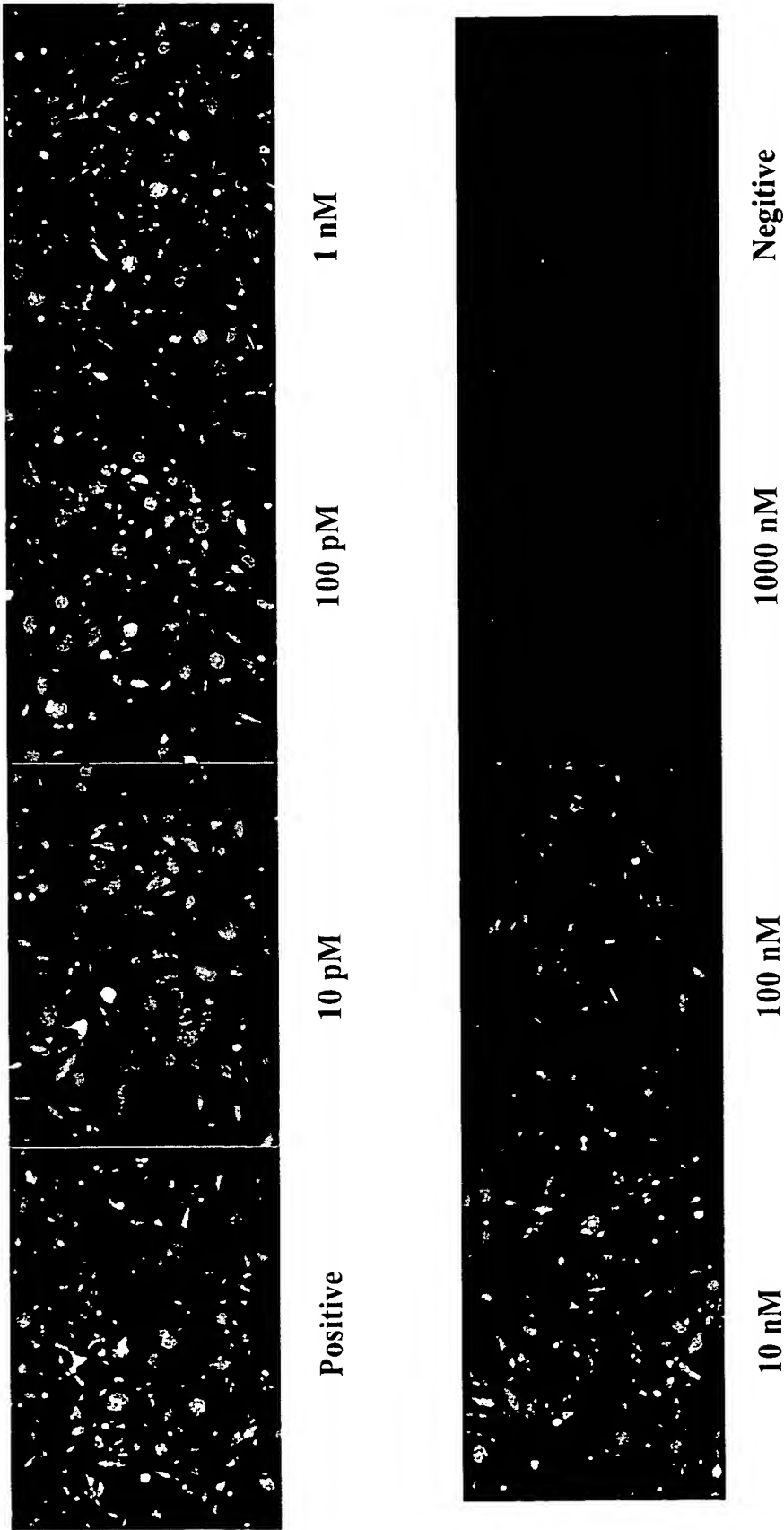
**Day 2      Change Media**



**Day 3-4      Read Plates by Fluorescent Micro-Plate Reader**

FIGURE 16

Phenotypic Antiretroviral Drug Resistant Test



**FIGURE 17A**

	Drug Name		Brand Name	Virologic Mean IC <sub>50</sub>	NIH IC <sub>50</sub> (Other Virus)	NIH IC <sub>50</sub> (III-B) (Other Cells)	GenPhar Reference: IC <sub>50</sub> Values		Patient	
	Drug	CS	Drug	(CND0)	PBMC					
										G
uM	NRTI	Zidovudine	Retrovir	0.03	0.05	0.05	0.0008	IC <sub>50</sub> Shift	0.026	ZDV
		Didanosine	Videx	4.57	0.46	8	3.03	IC <sub>50</sub> Shift	26.3	ddl
		Stavudine	Zerit	0.66	0.009	0.05	0.077	IC <sub>50</sub> Shift	5.5	d4T
		Zalcitabine	Hivid	0.68	0.011	0.3	0.115	IC <sub>50</sub> Shift	0.22	ddC
		Lamivudine	Epivir	1.77	>100		0.404	IC <sub>50</sub> Shift	201.5	3TC
NRTI	Abacavir	Ziagen	1.74		4	0.827	IC <sub>50</sub> Shift	9.2	ABC	
	Tenofovir	Viread		0.04	8.5	10.3	IC <sub>50</sub> Shift	22.3	TNFV	
nM	NNRTI	Nevirapine	Viramune	78		48	45.95	IC <sub>50</sub> Shift	14,300	NVP
		Delavirdine	Rescriptor	32	0.0001	0.1		IC <sub>50</sub> Shift	311.2	DLV
		Efavirenz	Sustiva	2	0.003	1.575	1.01	IC <sub>50</sub> Shift	55.7	EFV

FIGURE 17B

	Drug Name      Brand Name		Virologic Mean IC <sub>50</sub>	NIH IC <sub>50</sub> (Other Virus)	NIH IC <sub>50</sub> (III-B)  (Other Cells)	GenPhar Reference: IC <sub>50</sub> Values		Patient		
								JL	G	
								Drug		
uM	NRTI	Zidovudine	Retrovir	0.03	0.05	0.05	0.0008	IC <sub>50</sub> Shift	0.074 92.5	ZDV
		Didanosine	Videx	4.57	0.46	8	3.03	IC <sub>50</sub> Shift	57.8 19.07591	ddl
		Stavudine	Zerit	0.66	0.009	0.05	0.077	IC <sub>50</sub> Shift	2.3 29.87	d4T
		Zalcitabine	Hivid	0.68	0.011	0.3	0.115	IC <sub>50</sub> Shift	0.4 3.48	ddC
		Lamivudine	Epivir	1.77	>100		0.404	IC <sub>50</sub> Shift	6.2 15.35	3TC
		Abacavir	Ziagen	1.74		4	0.827	IC <sub>50</sub> Shift	10.3 12.45	ABC
NNRTI	Tenofovir	Viread		0.04	8.5	10.3	IC <sub>50</sub> Shift	23.5 2.28	TNFV	
	Nevirapine	Viramune	78		48	45.95	IC <sub>50</sub> Shift	98,700 2148	NVP	
nM	NNRTI	Delavirdine	Rescriptor	32	0.0001	0.1		IC <sub>50</sub> Shift		DLV
		Efavirenz	Sustiva	2	0.003	1.575	1.01	IC <sub>50</sub> Shift	127.1 125.8	EFV

FIGURE 17C

	Drug Name      Brand Name		Virologic Mean IC <sub>50</sub> (CNDQ)	NIH IC <sub>50</sub> (Other Virus)	NIH IC <sub>50</sub> (III-B) (Other Cells)	GenPhar Reference: IC <sub>50</sub> Values		Patient	
								JM	Drug
								G	
uM	NRTI	Zidovudine	Retrovir	0.03	0.05	0.0008	IC <sub>50</sub> Shift	0.042 52.5	ZDV
		Didanosine	Videx	4.57	0.46	3.03	IC <sub>50</sub> Shift	8.3 2.74	ddl
		Stavudine	Zerit	0.66	0.009	0.077	IC <sub>50</sub> Shift	4.6 59.74	d4T
		Zalcitabine	Hivid	0.68	0.011	0.115	IC <sub>50</sub> Shift	0.036 0.31	ddC
		Lamivudine	Epivir	1.77	>100	0.404	IC <sub>50</sub> Shift	0.26 0.64	3TC
		Abacavir	Ziagen	1.74		0.827	IC <sub>50</sub> Shift	2.4 2.9	ABC
		Tenofovir	Viread		0.04	10.3	IC <sub>50</sub> Shift	13.6 1.32	TNV
		Nevirapine	Viramune	78		45.95	IC <sub>50</sub> Shift	273 5.94	NVP
		Delavirdine	Rescriptor	32	0.0001		IC <sub>50</sub> Shift		DLV
		Efavirenz	Sustiva	2	0.003	1.01	IC <sub>50</sub> Shift	37.4 37.03	EFV
nM	NNRTI								

FIGURE 17D

Patient IC <sub>50</sub> , Shift, and R <sup>2</sup> Values for GenPhar Tru-Select (G) and Virlogic Phenosense (V) Assay Tests																	
Drug Name	Brand Name	Virologic Mean IC <sub>50</sub> (CNDOL)	NIH IC <sub>50</sub> (Other Virus) PBMC	NIH IC <sub>50</sub> (III-B) (Other Cells)	GenPhar III-B IC <sub>50</sub> Values	CS		JL		JM		Drug					
						G	V	G	V	G	V						
nM	PI	Amprenavir	Argenrase	12		7	34.068	IC <sub>50</sub>	934		927		93.03		2.73071504		APV
								Shift	27.415757		27.21029		0.90				
								R <sup>2</sup>	0.86		0.78						
		Indinavir	Crixivan	6			35.833	IC <sub>50</sub>	242.8		817.9		36.61		1.02		IDV
								Shift	6.775877		22.82533		0.93				
								R <sup>2</sup>	0.96		0.84						
		Lopinavir	Aluviran					IC <sub>50</sub>	#DIV/0!		#DIV/0!		#DIV/0!				LPV
								Shift									
								R <sup>2</sup>									
		Nelfinavir	Viracept	3		10	34.026	IC <sub>50</sub>	584.7		945.6		16.41				NFV
								Shift	17.18		27.79		0.48				
								R <sup>2</sup>	0.97		0.80		0.96				
		Ritonavir	Norvir	14		46	21.384	IC <sub>50</sub>	955.7		1151		384.5				RTV
								Shift	44.7		53.83		17.98				
								R <sup>2</sup>	0.83		0.85		0.92				
		Saquinavir	Inivase	3			34.327	IC <sub>50</sub>	124.6		824.7		33.56				SQV
								Shift	3.63		24.02		0.98				
								R <sup>2</sup>	0.92		0.83		0.95				



FIGURE 18A

# Dose Response & IC<sub>50</sub> Shift Curves for ZDV

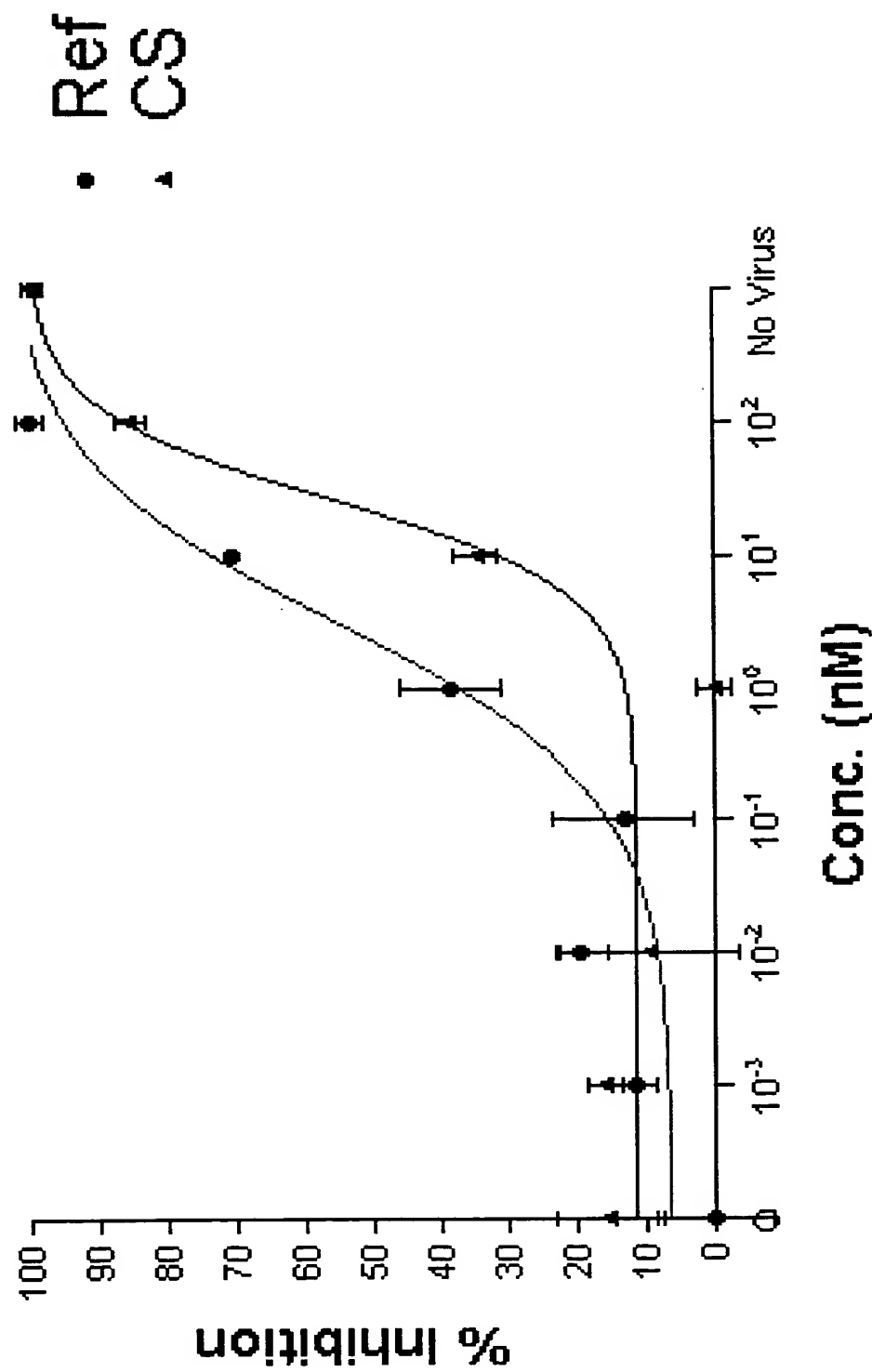


FIGURE 18B

# Dose Response & IC<sub>50</sub> Shift Curves for NVP

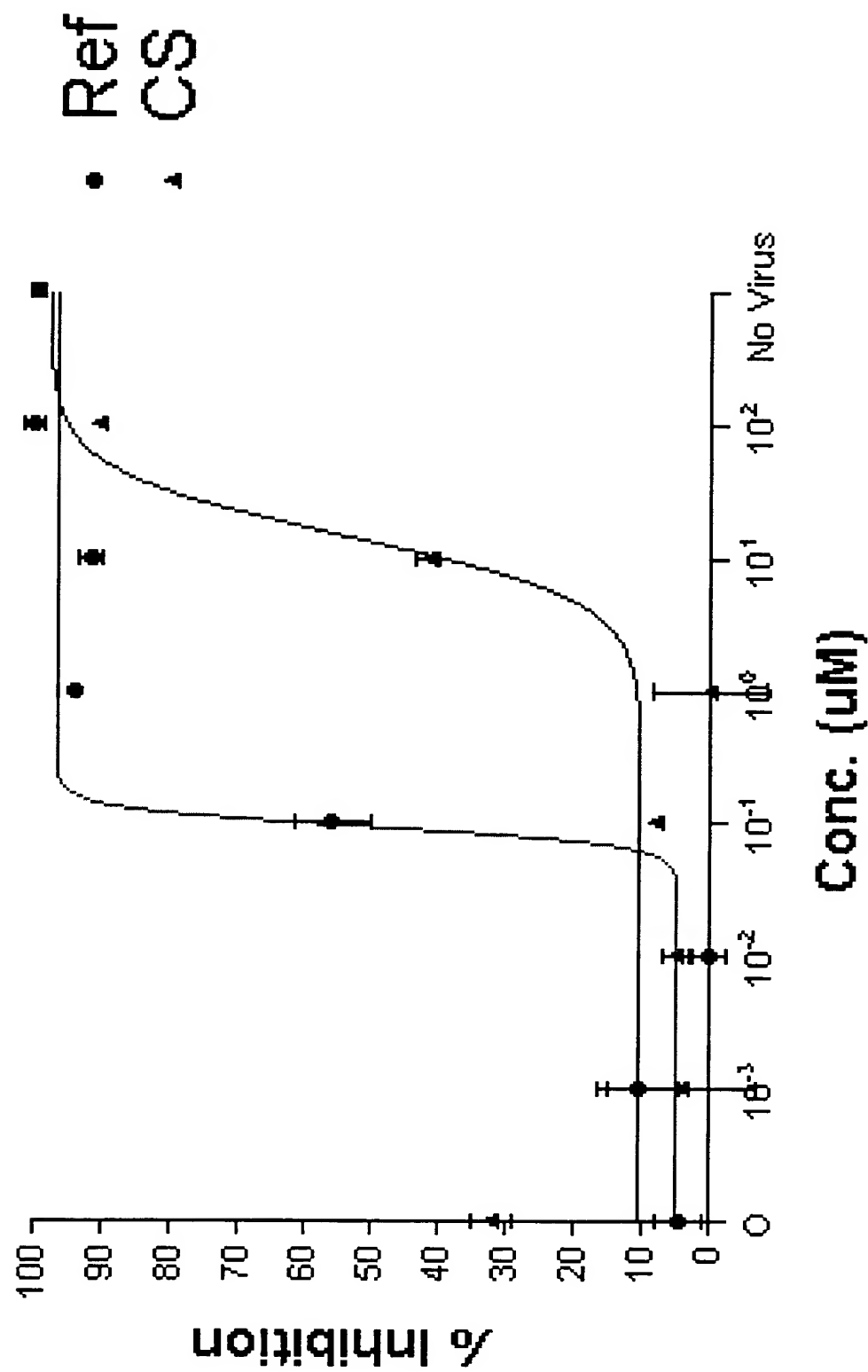


FIGURE 18C

## Dose Response & IC<sub>50</sub> Shift Curves for RTV

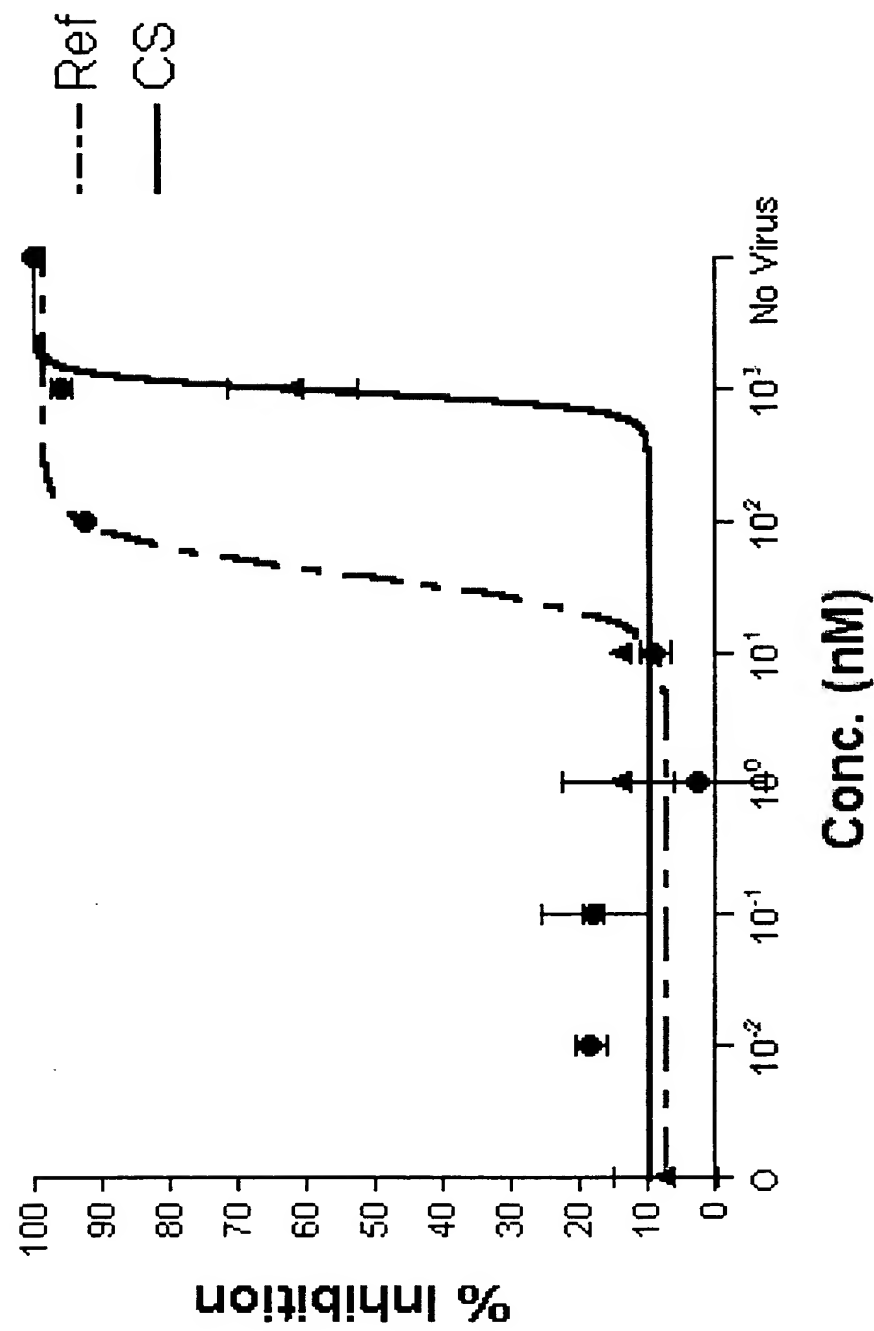
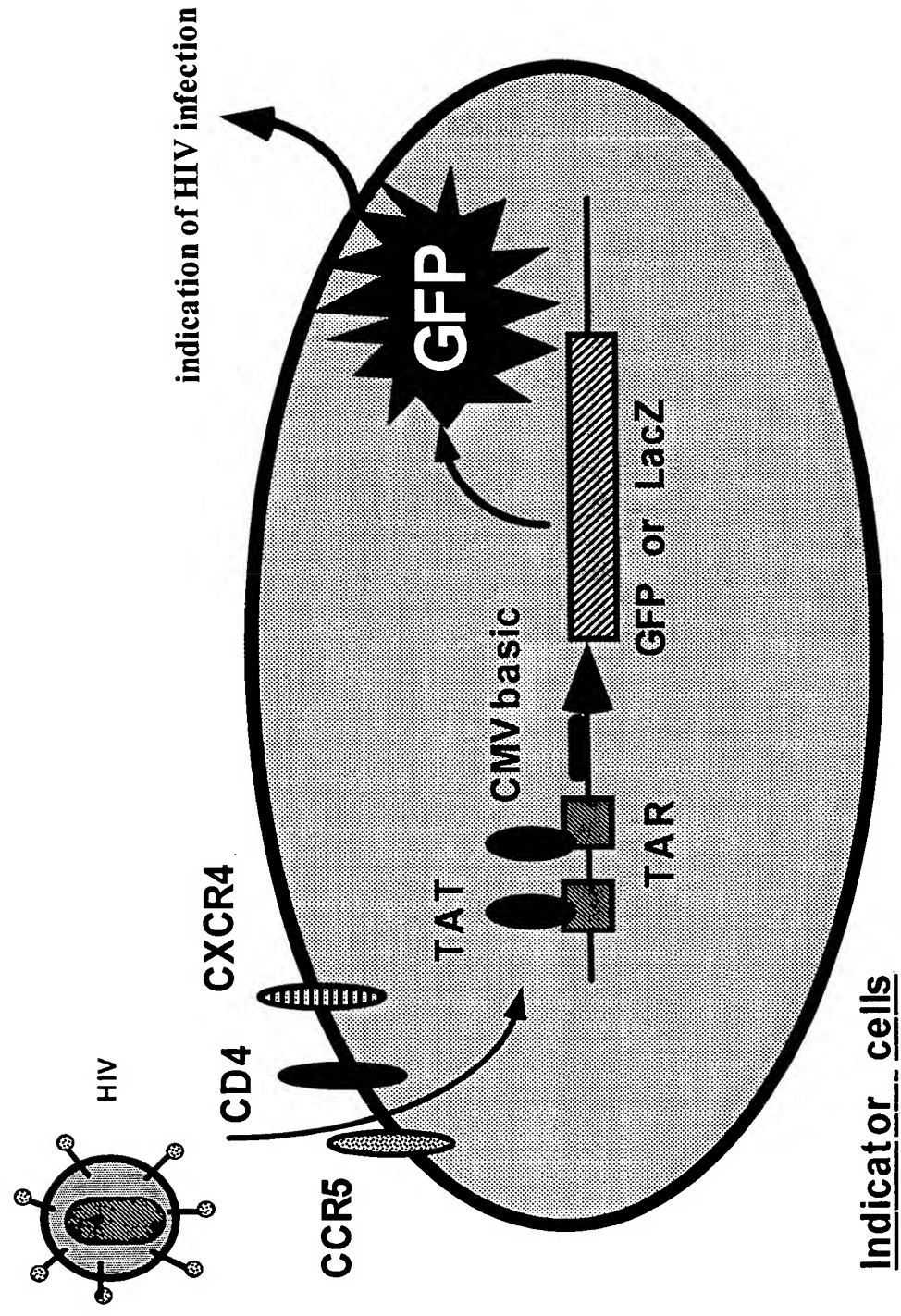


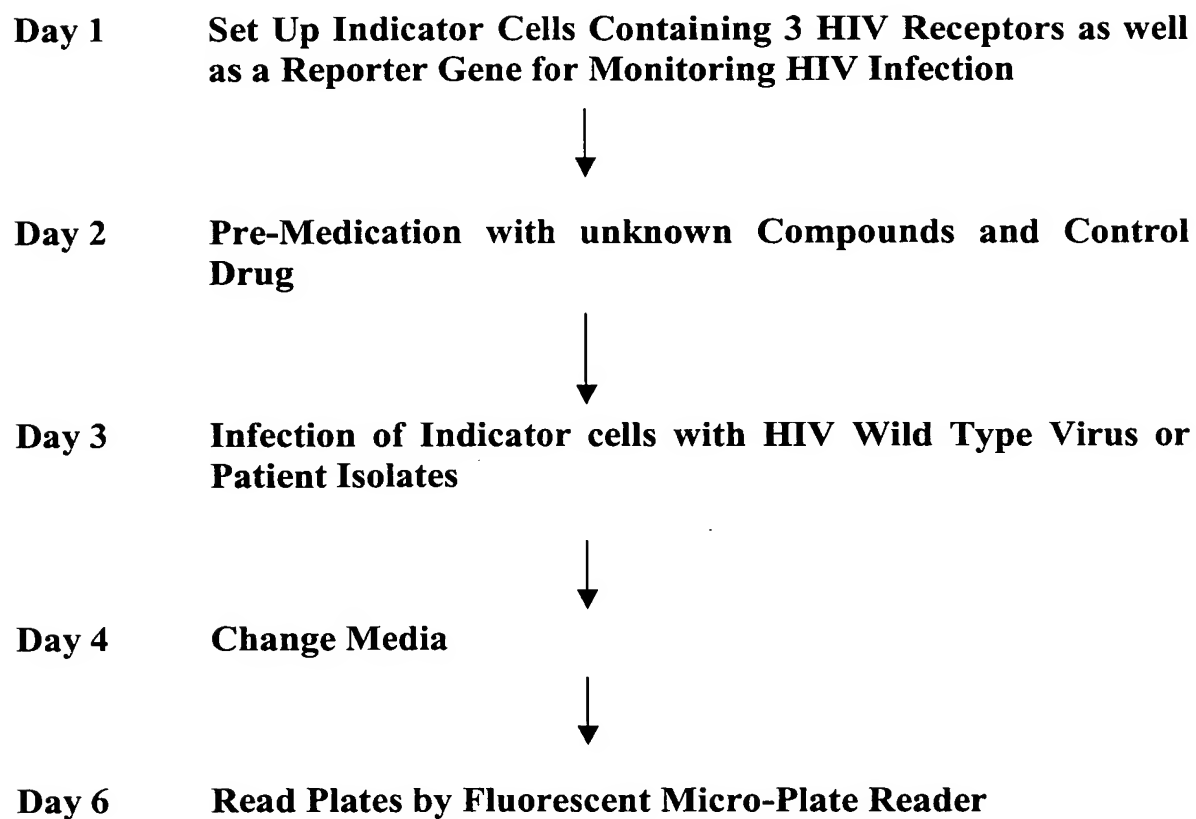
FIGURE 19

Mechanism of the HIV infection indicator cells



**FIGURE 20**

**HIV DRUG SCREENING ASSAY FOR ESI**



## **FIGURE 21**

### **HIV DRUG SCREENING ASSAY FOR LSI**

#### **PRIMARY PLATE**

**Set Up Infection Plate of Cells Containing 3 HIV Receptors  
with HIV in the Presence of a Test Agent  
for a Suitable Period of Time**

#### **SECONDARY PLATE**

**- Day 1      Set Up Indicator Cells Containing 3 HIV Receptors as well  
as a Reporter Gene for Monitoring HIV Infection**



**Day 1      Transfer the Supernatant of the Culture in the Primary  
Plate to the Indicator Cells for Titration of HIV**



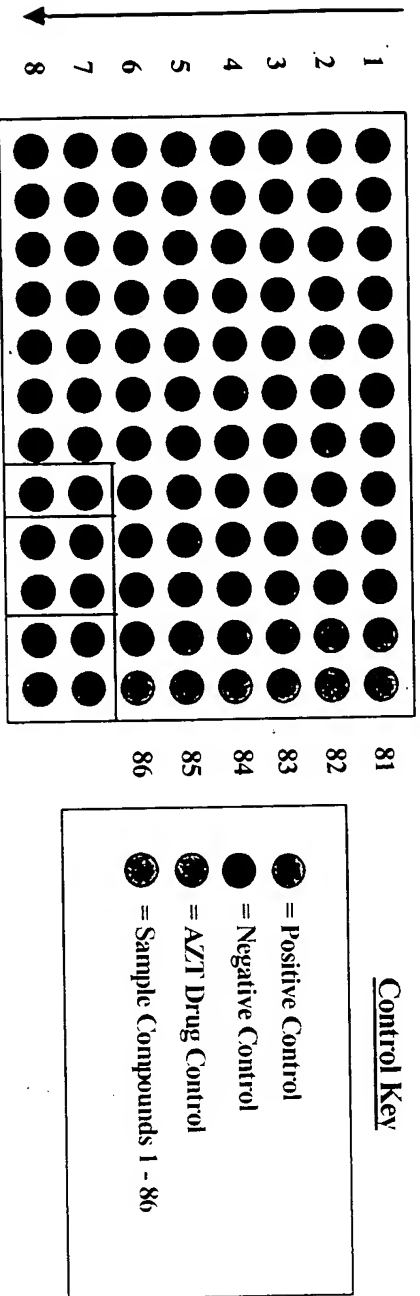
**Day 2      Change Media**



**Day 3-4      Read Plates by Fluorescent Micro-Plate Reader**

FIGURE 22

Phase I Layout



**FIGURE 23**

## Phase II Layout

**(Antiviral assay / Cytotoxicity assay)**

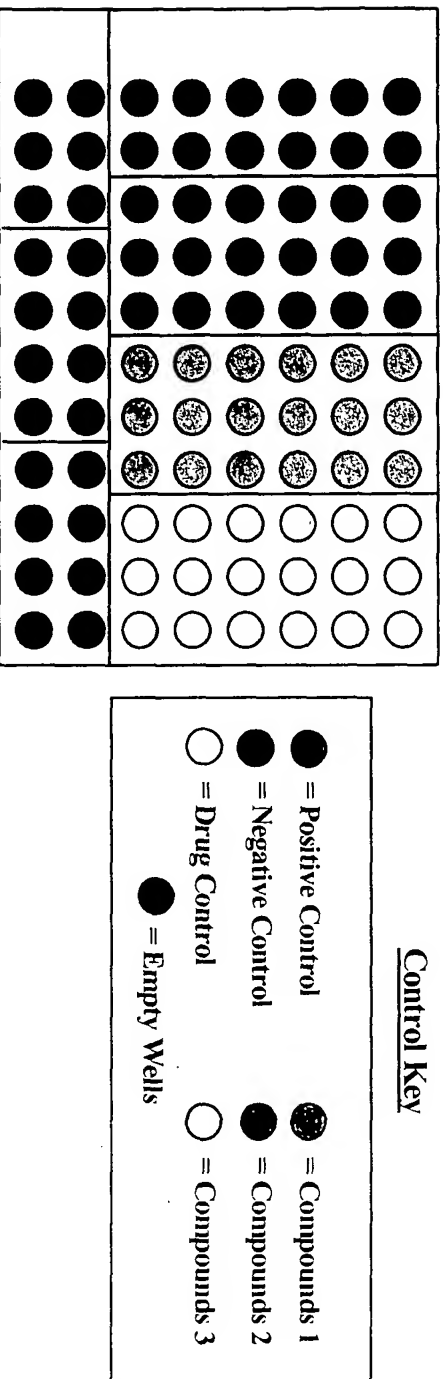




FIGURE 24

Phase III Antiviral Test Layout (WT vs Resistant HIV-1)

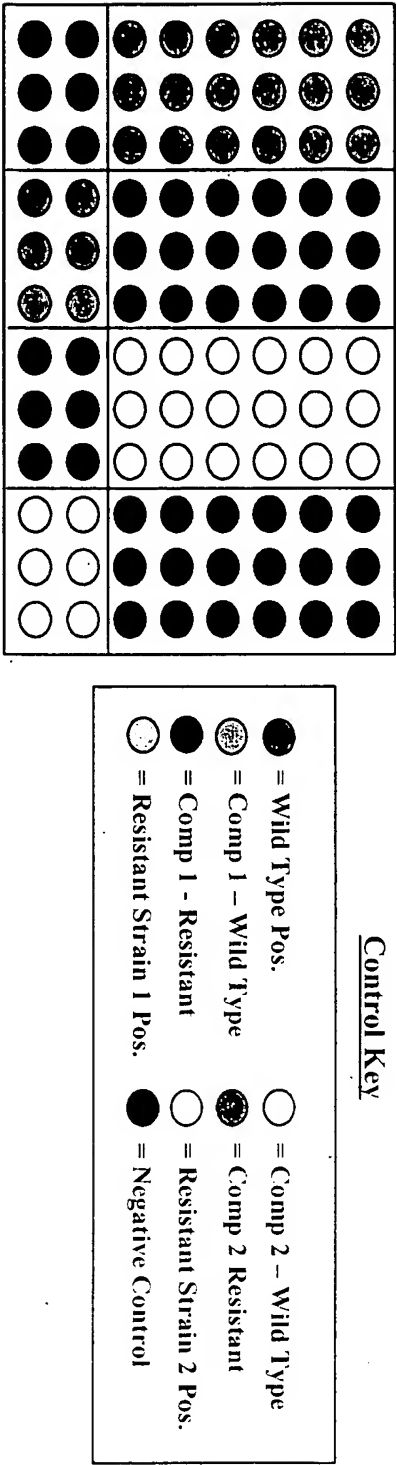


FIGURE 25

Phase I ESI Results

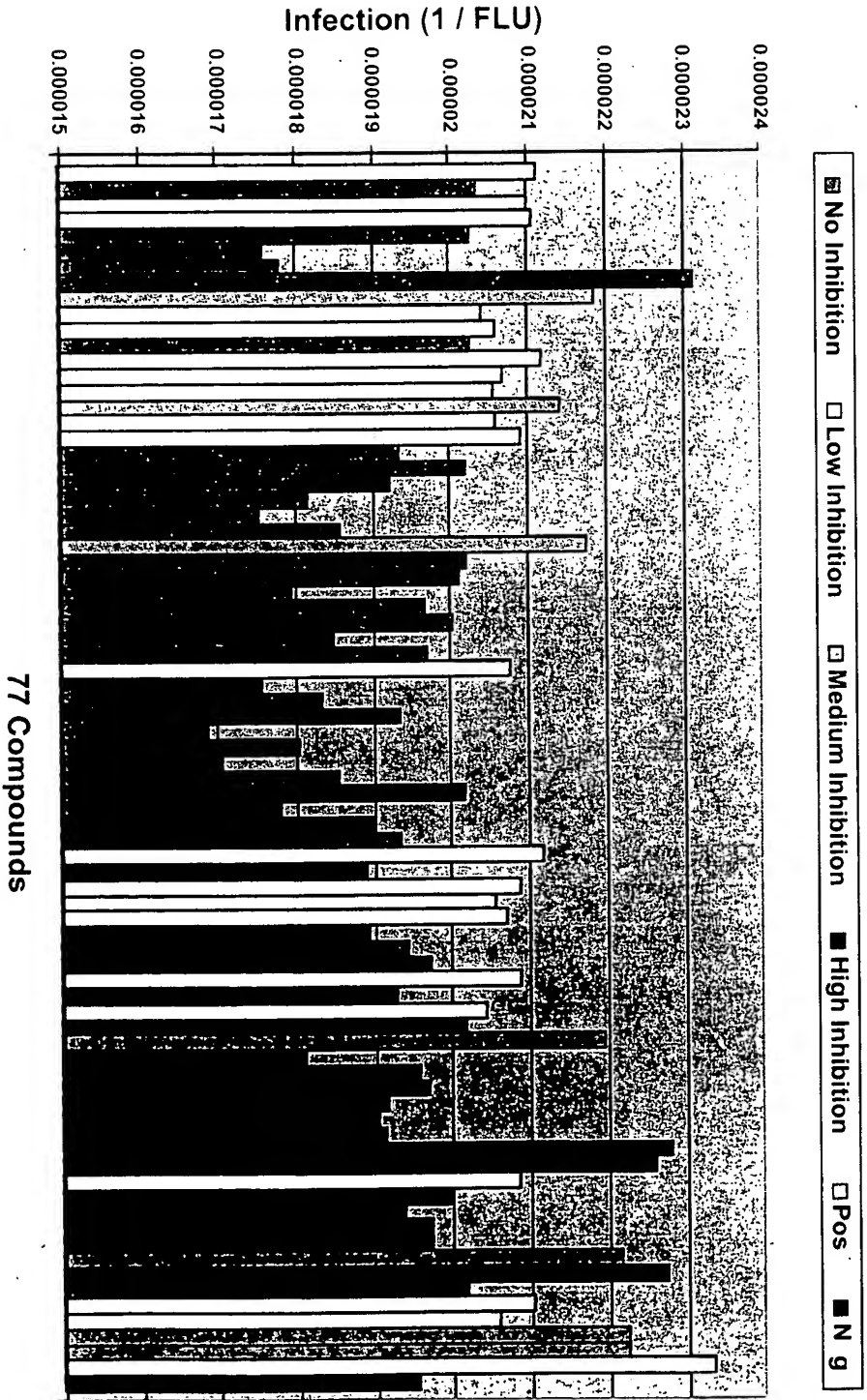


FIGURE 26

Phase I LSI Results

